REMARKS

Claims 1-24 are pending in the present application. Claims 15-24, have been withdrawn as being directed to non-elected subject matter. Claims 1-14 are rejected.

Claim 1, drawn to SEQ ID NO: 4 was withdrawn from consideration as being directed to a non-elected invention. Applicants have amended claim 1 to removed reference to "SEQ ID NO: 4" and respectfully request examination of amended claim 1.

The rejection of claims 1-14 under 35 U.S.C. § 112, first paragraph, as being indefinite is withdrawn. The rejection of claims 1-3, 7 10, 14 under 35 U.S.C. § 112, second paragraph, as being indefinite are withdrawn. The rejection of claims 1-3, 7-9, 11 and 13 under 35 U.S.C. § 102(b) as being anticipated by Lemanski *et al.*, (1996 *Biochem. Biophys. Res. Comm.* 229:974-981) is withdrawn.

Maintenance of Objections and Rejections

Claim 10, is rejected under 35 U.S.C. § 102(b) as being anticipated by Lemanski *et al.*, (1996 *Biochem. Biophys. Res. Comm.* 229:974-981).

Applicants respectfully traverse. However, in order to expedite prosecution, Applicants have amended claim 10 to recite "as identified by SEQ ID NO: 5." Applicants deem that this amendment overcomes the Examiner's rejection.

As such, claim 10 is allowable under 35 U.S.C. § 102(b) in the instant rejection. In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection

The Examiner has objected to the figure descriptions of Figures 3, 5 and 8. Applicants have amended the description to include the SEQ ID NOs in the description. Figure 5 has been amended to show that the two structures are represented by Figure 5A (normal) and Figure 5B (mutant). The figure was inadvertently referred to as Figure 5 only. No new matter has been added by virtue of these amendments and their entry is respectfully requested.

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Claims 1, 7, 10, 13 were objected to as the claims depicted the notation "SEQ ID NO.: 1" instead of 'SEQ ID NO: 1." Appropriate correction has been made.

In view thereof, Applicants respectfully request withdrawal of the instant objections.

Claims 1-14 are rejected under 35 U.S.C. § 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility.

Applicants respectfully traverse.

Applicants submit that the invention is useful for treating cardiac disease, for example. Applicants discuss, on page 1, lines 14-15, that the MIR molecules are essential for the "establishment and maintenance of normal heart cell function" and "survival of all higher organisms." Applicants further elaborate on the utility of the MIR molecules on page 2, lines 14-18:

> As the elderly population increases, and as larger segments of the population are subjected to high-stress employment situations, **cardiovascular disease is becoming increasingly prevalent.** The need for development of new therapeutic methods for **restoring heart muscle cells** continues to rise. Promising technologies of the future, such as replacement of damaged heart cells with cardiac cells grown in culture, are dependent upon finding the molecular keys to inducing a cardiac muscle cell phenotype. (Emphasis added).

Applicants therefore, disclose that the molecules are useful in treating heart disease by restoring heart muscle. The functional activities of the MIR-encoding nucleic acids, disclosed in the instant invention, include: binding to MIR proteins, induction of rhythmic contraction, myofibrillar induction, induction of differentiation of a cell into cardiac muscle phenotype.

The functional activities associated with the MIR encoding nucleic acids are summarized by applicants on page 9, lines 1-15:

> Myofibrillogenesis inducing RNA (MIR) is an RNA molecule expressed in embryonic endoderm, with the ability to induce formation of myofibrils in differentiating cardiomyocytes of normal, but not mutant individuals, in an animal model of heart development.
>
> In studies disclosed herein, the full-length nucleotide sequence of MIR is disclosed. It is further shown that MIR extracted from adult mammalian (sheep) heart has the ability to **promote ("rescue") heart cell differentiation** in mutant salamanders, enabling these cells to exhibit **normal rhythmic contractions, tropomyosin distribution, and myofibril formation.** Detection of **RNA-protein interactions** by Northwestern blotting and gel-shift assays further led to the isolation of two MIR-binding proteins having molecular weights (MW) of about 13-15 kDa and about 28-30 kDa. Comparison of MIR DNA sequences from normal and mutant embryos revealed a point mutation in the mutant DNA that resulted in the loss of functional (rescue) ability of the RNA, coupled with inability to bind the larger MW MIR-binding protein. Taken together, these results demonstrate that myofibrillogenesis and promotion of a normal cardiac muscle phenotype can be achieved through the interaction of **MIR RNA with one or more MIR-binding proteins.** (Emphasis added).

Furthermore, applicants teach on page 9, lines 13-15 that the results obtained "demonstrate that **myofibrillogenesis** and promotion of a normal cardiac muscle phenotype can be achieved through the interaction of MIR RNA with one or more MIR-binding proteins." Applicants also teach that the MIR molecules specifically bind to proteins which induces a cardiac muscle phenotype for the treatment of heart disease. See for example, page 14, lines 9-28, through to page 15, lines 1-8:

> The **MIR molecules** of the invention have been shown to **specifically binds** to MIR-binding proteins present in cells undergoing differentiation to a cardiac muscle phenotype. **The discovery of the interaction of MIR with MIR-binding proteins holds great promise for inducing a cardiac muscle phenotype in a cell through use of MIR and its interacting proteins.** Any protein can be used that specifically binds to MIR, leading to the induction of a cardiac muscle phenotype in a cell containing that protein. MIR-binding proteins can be isolated and identified by techniques known in the art, and further described herein. MIR-binding proteins in a cell or tissue can be separated for example in a first step by two-dimensional polyacrylamide gel (2D gel) electrophoresis, one of the most powerful methods to resolve complex protein mixtures. Although 2D gels are currently a widely used separation tool, reverse phase HPLC, capillary electrophoresis, isoelectric focusing and related hybrid techniques can also provide powerful means of resolving complex protein mixtures, and might also be used in the invention.
>
> In a second step, using a technique such as "Northwestern" blot analysis, the separated proteins can be tested for ability to bind to MIR, which is

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> generally labeled with a detectable substance. Where a radioactive label is used as a detectable substance, a MIR-binding protein of the invention may be detected by autoradiography. The results of the autoradiography reveal those proteins separated within the 2D gel, i.e., **"MIR-binding proteins," that display specific binding to the labeled MIR.** Quantitation of the binding can be achieved by various optical methods. Confirmation of the specificity of the RNA-protein binding can be accomplished using a method such as a gel shift assay, in which binding of the radiolabeled MIR RNA to the protein is competitively challenged with increasing concentrations of unlabelled ("cold") MIR. Disappearance of a radioactive band representing a **MIR:MIR-binding protein interaction in a dose-dependent manner is indicative of specific binding between the RNA and the protein.**
>
> In preferred embodiments of the method, MIR-binding proteins can have MWs of ~11-13 kDa and ~28-30 kDa. As shown in examples herein, alkaline MIR-binding proteins of these sizes were identified by Northwestern blotting using radiolabeled MIR as a probe of protein extracts from embryos undergoing cardiac morphogenesis. (Emphasis added).

As can be seen from the disclosure, an MIR-binding protein is a protein that binds to MIR. The MIR-binding proteins are characterized in the instant application as proteins that specifically bind to MIR and have been characterized by molecular weight of 11-13 kDa and 28-30 kDa. A detailed experimental protocol is disclosed in example 8, page 27, lines 13-27 through to page 28, lines 1-19. Results obtained are shown in Figures 6A and 6B. These MIR-binding proteins have not yet been named by applicants. Thus, applicants clearly teach how MIR-binding proteins function to " promote ('rescue') heart cell differentiation in mutant salamanders, enabling these cells to exhibit normal rhythmic contractions, tropomyosin distribution, and myofibril formation" and have identified these proteins based on specific binding to the MIR molecules.

In view thereof, applicants submit that claims 2 and 4 fully comply with 35 U.S.C. § 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 2 and 14.

In view of the foregoing, applicants submit that claims 1 and 14 complies fully with 35 U.S.C. § 101 and as such, these claims are allowable. In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 1-14 are rejected under 35 U.S.C. § 112, first paragraph. The Examiner rejects these claims asserting that they lack utility. Applicants respectfully traverse. Applicants submit that the invention is useful as discussed above. In view of thereof, Applicants respectfully request reconsideration and withdrawal of the instant invention.

Claims 1-14 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner rejects claims 1-2, 7 10, 13 due to the term "encodes." Applicants have amended these claims as per the Examiner's recommendations. Claims 6, 8-9, 11-12 and 14 were included in the rejection because they were dependent on claim 1. As such, these claims are allowable.

Claim 3 has been rejected as the Examiner considers the claim indefinite. Applicants have amended claim 3 to stand as an independent claim and includes the subject matter of claim 1. No new matter has been added by virtue of this amendment and its entry is respectfully requested.

Claim 4 was rejected as indefinite for reciting "greater than 166 nucleotides in length." Applicants have amended the claim to fully comply with 35 U.S.C. § 112, second paragraph.

Claim 5 has been withdrawn without prejudice. Applicants reserve the right to pursue the subject matter of claim 5 in one or more Continuation or Divisional applications.

The Examiner has rejected claim 7 "as it is unclear what significance can be attributed to an untranslated region of a polynucleotide sequence since it is not translated." Applicants respectfully traverse.

Applicants teach the significance and utility of such a molecule. For example, on page 3, lines 10-14:

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A portion of the purified nucleotide sequence that encodes a MIR molecule can include a first polynucleotide sequence that shares sequence identity with a second polynucleotide sequence within the **5' untranslated region of a second nucleic acid that encodes an RNA splicing factor.** In some embodiments, the RNA splicing factor is SmN. The first and second identical polynucleotide sequences can include the sequence of SEQ ID NO: 6. (Emphasis added).

On page 10, lines 26-28 through to page 11, lines 1-9, Applicants teach that the 5' untranslated region shares a 100% identity with :an RNA splicing factor:

In another aspect, the invention includes nucleic acids in the form of myofibrillogenesis-inducing ribonucleic acid (RNA) molecules (MIR) that are encoded by the DNA molecules of the invention and by definition are complementary to the DNA molecules. The RNA molecules of the invention are shown herein to have bioactive properties such as 1) inducing heart beating and myofibrillogenesis in the muscle cells of embryonic hearts and 2) binding to specific MIR-binding proteins. The latter interaction is thought to promote transcription of genes, such as tropomyosin, associated with muscle cell differentiation. Additionally, it is shown herein that a fragment of a MIR-encoding cDNA (i.e., SEQ ID NO:6) shares **100% identity with a sequence in the 5' untranslated region of the axolotl homolog of SmN, an RNA splicing factor** (Huntriss JD et al., 1993. Nucleic Acids Res. Aug 25;21(17):4047-53), **further supporting a role for MIR in regulation of muscle cell differentiation.** In preferred embodiments, the MIR molecules of the invention are between about 167 and about 620 nucleotides in length. (Emphasis added).

On page 32, lines 25-28 through to page 33, lines 1-12:

Example 16- Sequence Homology of MIR with a 5' Untranslated Region of axoSmN cDNA

In related studies, a full length cDNA encoding the axolotl homolog of the mammalian SmN, termed herein "axoSmN," was cloned. The mammalian SmN gene encodes a tissue-specific RNA slicing factor (Huntriss JD et al., 1993; Gerrelli D et al., 1994). Of note, comparison of the MIR cDNA sequence with the axoSmN sequence revealed an exact match of a portion of the MIR sequence (i.e., GCC GAT CCT TTG GAA TTT GTA CAT GTG ACC TCA AGG TTG CAC GCA TAT CCG AGC AGT TGC TGG ATT AGA GCA GGC ACT CCC TTG) (SEQ ID NO:6) with an identical sequence in the 5' untranslated region of the axoSmN gene. Referring to FIG. 7, the positions of these residues in the MIR cDNA sequence are

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indicated in italics. Referring to FIG. 8, a full-length cDNA sequence for axoSmN is shown. The underlined sequence in the 5'-untranslated region of axoSmN is the sequence exhibiting 100% sequence identity with a portion of the MIR cDNA. The shaded portion of the AxoSmN sequence represents a large deduced open reading frame showing homology to the mammalian SmN gene. Poly (A) tail and polyadenylation signal (in bold) are also indicated. **The finding of the common sequence in MIR and axoSmN points to a potential relationship between MIR and the axoSmN gene, possibly through common interacting proteins.** (Emphasis added).

Thus, Applicants teach the significance of the 5' untranslated region.

In view thereof, Applicants submit that claims 1-14 are allowable under 35 U.S.C. § 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 103

Claims 13-14 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Lemanski *et al.* (1996 *Biochem. Biophys. Res. Comm.* 229:974-981).

Applicants respectfully traverse, but to expedite prosecution, Applicants have amended the claims to recite "SEQ ID NO: 5." As the Examiner has acknowledged, Lemanski *et al.*, do not teach "a nucleic acid as identified by SEQ ID NO: 5."

Applicants reiterate that Applicants disclose a full length sequence of a MIR-encoding nucleic acid molecule (SEQ ID NO.: 5), including sequences that are equal to or greater than 166 nucleotides in length. Furthermore, applicants teach the importance of the secondary structure of the MIR-encoding molecule and functions associated therewith. (See above). Applicants teach the importance of a point mutation that destroys the functions of SEQ ID NO.: 5. See figure 5.

The sequences were not taught by Lemanski *et al.* and neither was their any disclosure or teaching as to hybridization conditions, the bioactivity of the MIR-encoding molecule related to specific nucleotide sequences. Although the sequence of clone #4 is embedded in the MIR-

encoding molecule, the sequence identified in Lemanski *et al.*, does not correspond to the sequence identified by the instant invention and **would not posses the secondary structure of the full length MIR-encoding molecule** as taught by Applicants.

Also, as discussed by applicants on page 33, lines 1-12, and shown in figure 8A, the SmN as represented by SEQ ID NO's.: 6 and 7 are not taught nor disclosed by Lemanski *et al.* SEQ ID NO's.: 6 and 7 do not match up to the clone #4 sequence shown in Figure 1 of Lemanski *et al.* In addition to the foregoing, the claims, as amended, precludes any teaching or suggestion by Lemanski *et al.*

It is respectfully submitted that for the foregoing reasons, claims 13 and 14 are patentable over the cited reference and satisfy the requirements of 35 U.S.C. §103. As such, these claims are allowable.

CONCLUSION

In view of the foregoing, reconsideration and withdrawal of all rejections and allowance of the application is respectfully solicited. Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no further extensions of time (beyond the one month petition) are required with submission of this paper, Applicants request that this submission also be considered as a retroactive petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

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Respectfully submitted,

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